Effects of *CCR5-Δ32* and *CCR2-64I* alleles on disease progression of perinatally HIV-1-infected children: an international meta-analysis

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Objective: Among perinatally infected children, the effects of certain alleles of the *CCR5* and *CCR2* genes on the rate of disease progression remain unclear. We addressed the effects of *CCR5-Δ32* and *CCR2-64I* in an international meta-analysis.

Methods: Genotype data were contributed from 10 studies with 1317 HIV-1-infected children (7263 person-years of follow-up). Time-to-event analyses were performed stratified by study and racial group. Endpoints included progression to clinical AIDS, death, and death after the diagnosis of clinical AIDS. The time-dependence of the genetic effects was specifically investigated.

Results: There was large heterogeneity in the observed rates of disease progression between different cohorts. For progression to clinical AIDS, both *CCR5-Δ32* and *CCR2-64I* showed overall non-significant trends for protection [hazard ratios 0.84, 95% confidence interval (CI) 0.58–1.23; and 0.87, 95% CI 0.67–1.14, respectively]. However, analyses of survival showed statistically significant time-dependence. No deaths occurred among *CCR5-Δ32* carriers in the first 3 years of life, whereas there was no protective effect (hazard ratio 0.95; 95% CI 0.43–2.10) in later years ($P = 0.01$ for the time-dependent model). For *CCR2-64I*, the hazard ratio for death was 0.69 (95% CI 0.39–1.21) in the first 6 years of life and 2.56 (95% CI 1.26–5.20) in

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subsequent years ($P < 0.01$ for the time-dependent model). *CCR5-Δ32* and *CCR2-64I* offered no clear protection after clinical AIDS had developed.

Conclusion: The *CCR5-Δ32* and *CCR2-64I* alelles are associated with a decreased risk of death among perinatally infected children, but only for the first years of life.

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Keywords: *CCR5-Δ32*, *CCR2-64I*, death, disease progression, HIV-1, perinatal transmission

Introduction

Host genetic factors are important determinants of the course of the disease among adults infected with HIV-1 [1–7]. Several genetic polymorphisms of chemokine and chemokine receptor genes have been proposed to be associated with slower or faster progression to AIDS or death. A substantial number of studies have addressed the 32-basepair deletion in the *CCR5* gene (*CCR5-Δ32*) [2] and the 64I mutation in the *CCR2* gene (*CCR2-64I*) [3]. A recent meta-analysis of individual participants' data from 17 studies of adults and children with hemophilia [7] demonstrated that carriers of these alleles experience delayed disease progression. These alleles have also been studied in children with perinatally acquired HIV-1 infection [8–17]. Although some data suggested delayed disease progression associated with *CCR5-Δ32*, other studies suggested no effect on disease progression or had inconclusive results [8–17]. There is also uncertainty about the effects of the *CCR2-64I* allele that have been evaluated in many of the same studies. Even large multicenter cohorts of perinatal HIV-1 infection lack the statistical power to detect modest, but clinically important, protective effects. Furthermore, in adults, the protective effects of these polymorphisms appear to be time-limited [18]. The examination of time-dependence in single perinatal cohorts is underpowered given the available sample sizes. We, therefore, conducted an international meta-analysis of individual participant data [19] with the contribution of pertinent data from several studies of perinatal HIV-1 transmission in Europe, Argentina, and the United States.

Methods

Organization of the meta-analysis

All research teams investigating the associations of genetic alleles with the course of HIV-1 disease progression in perinatally HIV-1-infected individuals were invited to contribute individual patient data to the meta-analysis. The teams were identified through MEDLINE searches (last update March 2001), abstracts of major meetings, communication with experts, an announcement in *Nature Medicine* [20], and presentations at AIDS meetings. The meta-analysis database remained open for the contribution of data and clarification of queries until June 2001.

Selection of databases, definitions and endpoints

We included both retrospective and prospective studies, so long as the mode of HIV-1 infection was ascertained to be perinatal transmission and children had been followed before 1 January 1997. Children infected through other ways were excluded. The documentation of perinatal infection was based on the criteria of each study, and typically involved a combination of virological assays with a lack of seroreversion. For both prospective and retrospective studies, the follow-up time for all children started at birth.

The major endpoints were clinical AIDS (stage C disease according to the 1994 revised CDC criteria) [21], death from any cause, and death after a clinical AIDS diagnosis. Immunological progression without clinical disease was not a study endpoint.

Follow-up in some studies continued during the late 1990s when subjects might have received highly active antiretroviral therapy (HAART; defined as combination treatment involving a protease inhibitor or three drugs including a non-nucleoside reverse transcriptase inhibitor). Although monotherapy and double nucleoside therapy generally have modest effects on HIV-1 disease progression, HAART would be expected to have a major effect [22], which may also be modified by specific genotypes [23]. In order to avoid the effects of HAART on the estimation of the effects of the genetic polymorphisms, we censored all follow-up at 1 January 1997. With very few exceptions, HAART was not used among children in the participating cohorts before that date. No data on *Pneumocystis carinii* or other prophylaxis were collected, but there is no evidence to suggest that chemokine receptor genotypes would interact with these prophylactic effects.

Participating teams conformed their data to the common definitions above. Errors in logic, internal inconsistencies, incompatibilities and missing data were discussed with the investigators of each team in an effort to correct them.

Statistical methods

CCR5-Δ32 is racially restricted to individuals of European descent [2], whereas the frequency of the *CCR2-64I* allele may vary by race [3]. Therefore, children in each study were separated into those of European descent (including Hispanic) and those of African descent. There were too few children of other racial descent to perform meaningful analyses thereof. Racial subgroups were entered as separate studies in the meta-analysis. *CCR5-Δ32* effects were only evaluated in children of European descent, whereas the *CCR2-64I* allele was examined in subjects of both European and African descent.

For *CCR5-Δ32*, we compared heterozygotes versus wild-type *CCR5* homozygotes; sensitivity analyses were limited to children who were also wild-type homozygotes for *CCR2,* whenever both polymorphisms had been genotyped. For *CCR2-64I*, we compared children with one or two copies of *CCR2-64I* versus wild-type *CCR2* homozygotes; sensitivity analyses were limited to wild-type *CCR5* homozygotes [7].

Analyses within each study and racial subgroup were initially performed using non-parametric methods with Kaplan–Meier plots [24]. Cox regressions were also conducted [25] to estimate study and race-specific hazard ratios. Heterogeneity in these hazard ratios was assessed with the Q statistic, considered significant for $P < 0.10$ [26]. In the absence of significant heterogeneity in the hazard ratios, synthesis of the data by random effects [26], fixed effects [26], or stratified Cox models ('pooled analysis') [27] yielded very similar results [27]. On the basis of our observations among HIV-1-infected adults [18], we hypothesized that the genetic effects of these polymorphisms in children might be greatest soon after infection and dissipate thereafter. In order to evaluate potential time-dependence, we used time-constant Cox models [25,27] on the basis of proportional hazards, as well as additional time-dependent models specifying different hazard ratios during different follow-up time intervals. The two-intervals models were specified by one cut-off point in time (at 1, 2, 3, 4, 5, 6, or 7 years) and the three-interval models were specified by two cut-off points in time (at 1 and 3, 1 and 4, 1 and 5, 1 and 6, 2 and 4, 2 and 6, 3 and 5, or 3 and 6 years). All Cox models were stratified by study and race.

If none of the time-dependent models was statistically significant at $\alpha = 0.05$, then the standard time-constant model was selected as the final model. If one or more time-dependent models fit significantly better than the time-constant model, we used the Akaike information criterion (AIC) to select the model that optimized goodness of fit and parsimony [18]. AIC is defined as the sum of -2 times the log likelihood plus two times the degrees of freedom. No three-interval models were selected by this criterion.

Epidemiological studies may be subject to various selection biases affecting the overall recorded rates of disease progression. Selection bias is minimized in studies that enrolled all eligible children at birth or retrospectively captured all HIV-1-infected children born in the participating institutions; or genotyped completely random samples of such populations of children. In contrast, studies that enrolled children at later ages may under-represent rapidly progressing children who died before they could be enrolled. A bias acting in the opposite direction could also occur in this setting, if children are referred (and included in a study), because of a more problematical disease course that reflects more rapid disease progression. The relative magnitude of the opposite biases may vary between studies, but the net effect of these potential selection forces may be deciphered by examining the overall rates of disease progression in each study. Therefore, we also performed analyses excluding studies in which the recorded rate of progression to stage C was less than 30% at the age of 6 years, i.e. substantially lower than what has been described for the incubation period of AIDS in vertical HIV-1 infection [28].

Analyses were conducted in SPSS (SPSS Inc., Chicago, IL, USA) and in SAS (SAS Institute, Cary, NC, USA). All *P*-values are two-tailed.

Results

The meta-analysis database

We obtained data on 1411 children enrolled in 10 different studies [8–17], but excluded two children who were not vertically infected and 92 children born after 1 January 1997. Among 1317 eligible children with vertical infection (Table 1), 992 children were of European descent, 320 children were of African descent, and five children were of other races. The median year of birth was 1991 (interquartile range 1988–1993). *CCR5-Δ32* genotyping had been performed in all 10 studies and data were available for 1285 of the 1317 children. *CCR2-64I* information was available for 915 children.

Among subjects of European descent, the allele frequency of *CCR5-Δ32* was 4.8%; the frequency was between 3.5 and 5.3% in all studies conducted in France, Italy, Spain or Argentina, whereas it was 7.7% in the National Cancer Institute (NCI) cohort (the largest US cohort), and 10.9% in children enrolled in four centers participating in the European Collaborative Study (ECS). There were only eight subjects with this polymorphism (overall rate 1.4%) in subjects of African

Table 1. Studies of perinatal transmission included in the meta-analysis.

Cohort name	N	Race E/A	AIDS	Death[a]	*CCR5*		*CCR2*		
					w/w	*w/Δ32*	*w/w*	*w/64I*	*64I/64I*
EPF	415	300/115	92	58 (46)	384	31	313	86	5
Argentina	348	348/0	149	8 (8)	303	27	225	67	10
NCI	127	71/56	70	30 (30)	115	12	ND	ND	ND
Milan/Rome	101	101/0	31	11 (11)	82	7	ND	ND	ND
Harlem	97	0/97	42	28 (22)	94	3	75	18	1
Padova	72	71/1	31	20 (18)	67	5	53	18	1
Spain	47	47/0	10	7 (2)	42	5	ND	ND	ND
Seattle	46	20/22	14	3 (3)	38	6	28	14	1
Northshore	40	11/28	5	0 (0)	39	1	ND	ND	ND
ECS centers	24	23/1	11	4 (4)	19	5	ND	ND	ND
Total	1317	992/320	456	169 (144)	1183	102	694	203	18

E/A, European/African descent; ECS, European Collaborative Study (genotyping performed on limited samples from four participating centers); EPF, Enquete Perinatale Francaise; NCI, National Cancer Institute; ND, no data; w, wild-type.
Some genotype splits may not add up to the total study N, because a few subjects were not genotyped for specific alleles in some cohorts.
[a]The numbers in parentheses refer to the number of deaths occurring after stage C (clinical AIDS stage C disease per 1994 CDC criteria) diagnosis.

descent, probably a result of some European descent parentage. The frequency of the *CCR2-64I* allele varied between 10.2 and 22.5% in studies of European descent children and between 10.6 and 16.1% in studies of African descent children (overall 13.1%).

For 19 children no clinical follow-up was available, leaving 1298 children for the analysis. Sensitivity analyses assuming the 19 children had no disease progression yielded similar results (not shown). A total of 456 children progressed to clinical AIDS, and 169 died (144 after clinical AIDS diagnosis) before 1 January 1997. Total follow-up time was 6117 person-years until clinical AIDS or censoring (median 4.17 years) and 7263 person-years until death or censoring (median 5.22 years) for *CCR5-Δ32* analyses, and 4114 and 4820 person-years, respectively, for *CCR2-64I* analyses (median 4.05 and 5.00 years).

Study design considerations

Studies differed substantially in how they had selected their genotyped population. No study selected children on the basis of genotype. The Enquete Perinatale Francaise (EPF) cohort [8,9] consisted of one population of consecutively prospectively enrolled children (thus likely to reflect a largely unselected cohort) and one population of children with variable follow-up starting at various ages and with overall very low rates of recorded disease progression. Similarly, the Argentina cohort [10] and the Harlem study [13] included one prospective, consecutive set of children enrolled at birth in one central hospital and one set of referred, prevalent HIV-1-infected children, but the disease progression rates were not as markedly different in the two subsets. The Padova study [11] was a prospective cohort of children enrolled at birth. The NCI study [12] consisted of children referred to the NCI at various ages. The ECS is a very large prospective multicenter cohort [14], but only a limited sample of children was genotyped. The Seattle study [15] was a retrospective study of children followed since birth in a single hospital. The Northshore study [16] and the Milan/Rome and Madrid/Barcelona studies [17] were retrospective cohorts of children followed from different ages.

Given these differences, there was large heterogeneity in the rates of progression to clinical AIDS between the included studies, with estimated rates at 6 years ranging between 8 and 77% (log-rank $P < 0.001$). An overall progression rate to clinical AIDS of over 30% at 6 years was seen in the genotyped children from centers participating in the ECS (66%), in both sub-cohorts of the Argentina cohort (77 and 45%), in both sub-cohorts in the Harlem study (46 and 40%), in the NCI study (43%), in the prospective consecutive component of EPF (34%), and the Padova study (32%). Combining these cohorts, there were 915 children, 379 progressions to clinical AIDS, and 140 deaths.

Genetic effects in specific studies

Among the two largest studies, EPF demonstrated a more prominent *CCR5-Δ32* protective effect, whereas the Argentina study demonstrated a more prominent *CCR2-64I* protective effect for progression to clinical AIDS, but the difference could have been caused by chance given the limited sample size of each study. For example, for *CCR5-Δ32* the hazard ratios were 0.75 and 1.17 in EPF and the Argentina study, respectively, but the corresponding 95% confidence intervals (CI) were 0.30–1.87 and 0.68–1.99. For *CCR2-64I*, the corresponding hazard ratios were 1.25 (0.66–2.38) and 0.71 (0.47–1.07). The CI were typically much wider in other studies. Generally, study-specific CI of the hazard ratios based on Cox models overlapped widely (not shown), and there was no significant between-study heterogeneity ($P > 0.10$ for all).

Pooled analysis: clinical AIDS

For progression to clinical AIDS, for both alleles proportional hazards models seemed appropriate based on the inspection of Kaplan–Meier plots, and none of the time-dependent models showed significant time–gene interactions (Table 2). Both *CCR5-Δ32* and *CCR2-64I* showed overall non-significant trends for protection with hazard ratios 0.84 (95% CI 0.58–1.23) and 0.87 (95% CI 0.67–1.14), respectively. The overall hazard ratio for *CCR5-Δ32* was 0.38 (95% CI 0.09–1.57), when limited to wild-type *CCR2* children (n = 517). The hazard ratio for *CCR2-64I* was 0.89 (95% CI 0.68–1.18), when limited to wild-type *CCR5* infants (n = 833). The results were similar, when restricted to studies with overall progression rates above 30% at 6 years; the hazard ratio for *CCR5-Δ32* was 0.87 (95% CI 0.59–1.29) and the hazard ratio for *CCR2-64I* was 0.77 (95% CI 0.58–1.03).

Pooled analysis: mortality

For both *CCR5-Δ32* and *CCR2-64I*, a protective effect against death was present that was time-dependent (Table 3). The model with the best AIC suggested that *CCR5-Δ32* conferred total protection against death for the first 3 years as no deaths occurred in children carrying this allele during this time period; however, there was no protection after this time (hazard ratio 0.95; 95% CI 0.43–2.10). For *CCR2-64I,* hazard ratios for the best-fit model were 0.69 (95% CI 0.39–1.21) in the first 6 years, and 2.56 (95% CI 1.26–5.20) after that time. When limited to wild-type *CCR5* children, the respective hazard ratios were 0.63 (95% CI 0.35–1.14) versus 2.51 (95% CI 1.20–5.22). Among all children who survived for 6 years, those with *CCR2-64I* may even be at increased risk of death from then on.

The results were similar when restricted to studies with overall progression rates over 30% at 6 years. There were no deaths among children with *CCR5-Δ32* in the first 3 years, whereas there was no clear protection after this time (hazard ratio 0.78; 95% CI 0.33–1.83). For *CCR2-64I*, hazard ratios were 0.53 (95% CI 0.29–0.98) for the first 6 years, and 2.17 (95% CI 1.04–4.55) subsequently.

Pooled analysis: death after AIDS

No clear effect was seen for death after clinical AIDS for any allele, although a modest protection for *CCR5-*

Table 2. Effect of *CCR5-Δ32* and *CCR2-64I* on time to clinical AIDS according to different models.

Model	Time period	Hazard ratio	95% CI	AIC	*P* value*
CCR5-Δ32					
Time-constant[a]	All follow-up	0.84	0.58–1.23	3016.113	NA
Time-dependent A	< 1 year	0.62	0.32–1.22	3016.744	0.24
	⩾ 1 years	1.00	0.63–1.58		
Time-dependent B	< 2 years	0.70	0.40–1.23	3017.744	0.34
	⩾ 2 years	1.01	0.61–1.68		
Time-dependent C	< 3 years	0.71	0.43–1.19	3017.039	0.30
	⩾ 3 years	1.07	0.61–1.88		
Time-dependent D	< 4 years	0.70	0.43–1.14	3016.269	0.19
	⩾ 4 years	1.23	0.66–2.29		
Time-dependent E	< 5 years	0.86	0.56–1.31	3018.070	0.84
	⩾ 5 years	0.78	0.33–1.82		
Time-dependent F	< 6 years	0.84	0.56–1.28	3014.112	0.97
	⩾ 6 years	0.83	0.32–2.12		
Time-dependent G	< 7 years	0.82	0.54–1.24	3017.987	0.72
	⩾ 7 years	0.99	0.38–2.58		
CCR2-64I					
Time-constant[a]	All follow-up	0.87	0.67–1.14	2856.835	NA
Time-dependent A	< 1 year	0.73	0.48–1.11	2857.557	0.26
	⩾ 1 years	1.00	0.70–1.41		
Time-dependent B	< 2 years	0.90	0.64–1.26	2858.755	0.78
	⩾ 2 years	0.83	0.54–1.28		
Time-dependent C	< 3 years	0.91	0.67–1.25	2858.548	0.59
	⩾ 3 years	0.78	0.47–1.29		
Time-dependent D	< 4 years	0.92	0.68–1.25	2858.218	0.43
	⩾ 4 years	0.73	0.41–1.30		
Time-dependent E	< 5 years	0.86	0.64–1.14	2858.737	0.75
	⩾ 5 years	0.96	0.49–1.90		
Time-dependent F	< 6 years	0.86	0.65–1.14	2858.767	0.79
	⩾ 6 years	0.96	0.43–2.14		
Time-dependent G	< 7 years	0.89	0.67–1.17	2858.631	0.65
	⩾ 7 years	0.69	0.24–1.98		

AIC, Akaike information criterion; CI, confidence interval; NA, not applicable.
* *P* value for change in −2 log likelihood compared with the time-constant model.
[a]This model with the lowest AIC is the best in terms of combining fit and parsimony.

Table 3. Effect of *CCR5-Δ32* and *CCR2-64I* on survival according to different models.

Model	Time period	Hazard ratio	95% CI	AIC	*P* value*
CCR5-Δ32					
Time-constant	All follow-up	0.56	0.26–1.20	925.024	NA
Time-dependent A	< 1 year	0	NE	924.603	0.12
	⩾ 1 years	0.67	0.31–1.45		
Time-dependent B	< 2 years	0	NE	923.242	0.05
	⩾ 2 years	0.74	0.34–1.62		
Time-dependent C[a]	< 3 years	0	NE	919.952	0.01
	⩾ 3 years	0.95	0.43–2.10		
Time-dependent D	< 4 years	0.15	0.02–1.09	922.747	0.04
	⩾ 4 years	1.02	0.43–2.43		
Time-dependent E	< 5 years	0.38	0.12–1.21	925.937	0.30
	⩾ 5 years	0.87	0.30–2.48		
Time-dependent F	< 6 years	0.33	0.10–1.05	924.587	0.12
	⩾ 6 years	1.17	0.40–3.42		
Time-dependent G	< 7 years	0.39	0.14–1.08	925.131	0.17
	⩾ 7 years	1.27	0.36–4.44		
CCR2-64I					
Time-constant	All follow-up	1.06	0.70–1.63	1003.207	NA
Time-dependent A	< 1 year	0.79	0.29–2.12	1004.745	0.50
	⩾ 1 years	1.14	0.72–2.47		
Time-dependent B	< 2 years	0.50	0.21–1.19	1000.059	0.02
	⩾ 2 years	1.50	0.91–2.47		
Time-dependent C	< 3 years	0.53	0.25–1.13	998.505	0.01
	⩾ 3 years	1.71	1.00–2.93		
Time-dependent D	< 4 years	0.65	0.34–1.25	1000.21	0.03
	⩾ 4 years	1.73	0.97–3.09		
Time-dependent E	< 5 years	0.68	0.37–1.23	999.215	0.01
	⩾ 5 years	1.99	1.06–3.75		
Time-dependent F[a]	< 6 years	0.69	0.39–1.21	997.129	< 0.01
	⩾ 6 years	2.56	1.26–5.20		
Time-dependent G	< 7 years	0.93	0.57–1.51	1003.725	0.22
	⩾ 7 years	1.74	0.73–4.76		

AIC, Akaike information criterion; CI, confidence interval; NE, not estimable; NA, not applicable.
**P* value for change in −2 log likelihood compared with the time-constant model.
[a]This model with the lowest AIC is the best in terms of combining fit and parsimony.

Δ32 heterozygotes could have been missed because of limited data. There were six deaths among 30 children with *CCR5-Δ32* versus 85 deaths among 277 wild-type *CCR5* children (20 versus 30.7%; stratified $P = 0.44$). There were 20 deaths among 63 children with *CCR2-64I* versus 70 deaths among 210 wild-type *CCR2* children (31.7 versus 33.3%; stratified $P = 0.35$).

Discussion

This international meta-analysis contributes to understanding the potential effects of *CCR5-Δ32* and *CCR2-64I* alleles on the rate of disease progression among perinatally HIV-1-infected children. Both alleles were associated with a decreased mortality risk in the first years of life, but in subsequent years the protective effect disappeared, and in the case of *CCR2-64I* reversed. Results were less conclusive regarding progression to clinical AIDS. Neither allele had a clear-cut protective effect once clinical AIDS developed.

The results of this meta-analysis largely agree with findings of a recent meta-analysis of mostly adult's cohorts [7]. The results are statistically less conclusive in the perinatal meta-analysis, given the smaller sample size. Moreover, whereas the non-perinatal meta-analysis included mostly cohorts of northern European descent, the perinatal meta-analysis largely included cohorts of southern European descent, in whom the prevalence of *CCR5-Δ32* is lower, and thus the power to show statistically significant differences between *CCR5* genotypes is reduced. Furthermore, although the perinatal cohorts have the advantage of a fairly exact knowledge of the time of infection, strong selection forces (in the form of early infant deaths) could be operating, especially in retrospective studies and those without consecutive enrollment, favoring overall the inclusion of slow progressors. The progression rates in the studies included were highly heterogeneous. Differences in study designs would allow for selection biases to operate in variable degrees upon the choice of enrolled children. Cohorts with a large selection bias in favor of slow progressors may tend to show smaller protective effects for *CCR5-Δ32* and *CCR2-64I*, if these two alleles are more influential in the early years of the infection but lose their importance in more long-standing disease. Nevertheless, the same conclusions were reached when the meta-analysis was limited to the cohorts that seemed to have less net selection bias in favor of slow progressors. Finally,

publication lag and bias [29,30] is often a concern in meta-analyses, when studies with 'negative' results tend to remain unpublished. However, our search was exhaustive and our communication with many experts in the field should have minimized this concern.

Acknowledging these caveats, in both meta-analyses, protective effects were seen for both *CCR5-Δ32* and *CCR2-64I*. The dynamics of the genetic influences of these alleles may be qualitatively similar in both children and adults. There is strong experimental evidence to support the protective role of *CCR5-Δ32*, because CCR5 is a major co-receptor of HIV-1 [31]. The biological rationale for the protective effect of *CCR2-64I* is still elusive [32]. It is unclear whether it may reflect linkage disequilibrium with some other allele or a direct genetic effect.

We found evidence that the protective effect of *CCR5-Δ32* and *CCR2-64I* may be time-limited, but these time-dependent relative risks should be interpreted with some caution. In the meta-analysis of non-perinatal cohorts of seroconverters, time-dependent effects were also discerned [18]. For *CCR5-Δ32*, the protection was apparently lost at 2 years after the development of clinical AIDS, whereas for *CCR2-64I*, the protection was reduced at 4–8 years after seroconversion, and was completely dissipated with longer follow-up with no protection against death after AIDS. The HIV-1 incubation period is shorter [28] and the genetic protection may be lost earlier in children than in adults. This meta-analysis suggests that the biological mechanism of the protection, conferred in particular by *CCR2-64I*, may relate to the interaction of the virus with the host during the first few years of the infection. The finding of a potentially deleterious role of *CCR2-64I* on mortality after 6 years is interesting, and unless it represents a spurious overfit to the available data, it may suggest that children with *CCR2-64I* may suffer a disadvantage in the long term. More research would be needed to develop a biological mechanism for this possible disadvantage.

There is some evidence that HIV-1 perinatal transmission is linked to viral tropism for *CCR5*, and that most neonates are infected with viruses that are capable of using only the *CCR5* receptor [33,34]. Even in mothers who harbor *CCR5*-tropic viruses that can use alternative co-receptors (such as CXCR4), the strain eventually infecting the infant may still lack T-cell line tropism and may only be able to use *CCR5* [35,36]. One study [17] has shown that children carrying *CCR5-Δ32* may exhibit especially rapid depletion of CD4 cells if the virus strain is tropic for the MT-2 cell line. Such strains use the CXCR4 receptor (X4 viruses) [37]. A similar interaction between viral phenotype and host genotype has also been described by other investigators [38]. Although we did not collect viral phenotype data in this meta-analysis, it has been reported that early disease progression is mainly associated with an R5 type isolate [11]; thus the protective effect of *CCR5-Δ32* (and potentially also *CCR2-64I*) may be lost with the emergence of viruses with an extended co-receptor usage. Perhaps disease progression is accelerated disproportionately once X4 HIV-1 strains have developed in infected children with *CCR5-Δ32*. Surrogate markers would also be useful to analyse in future studies, to clarify whether the host genetic effects upon disease progression are mediated through adjustment of the viral setpoint and modification of the rate of CD4 cell loss [7,9,17].

The meta-analysis highlights the difficulty of assessing the effects of modest, but clinically significant, magnitude in the field of perinatal transmission. Previously published results from some of the cohorts included in this meta-analysis may have seemed contradictory. The largest cohort, EPF, had shown [8] a significantly protective effect for *CCR5-Δ32*, whereas smaller cohorts had mostly shown non-significant 'negative' results. The original studies used diverse definitions for disease progression (clinical or immunological disease progression), whereas the meta-analysis had the advantage of using a more strict, common definition across all studies. In addition, the original findings from smaller cohorts were statistically imprecise. Even multicenter cohorts are underpowered to detect hazard ratios in the range of 0.70–0.80, and may have insufficient power even to detect hazard ratios in the 0.30 range. For having 80% power (at the 0.05 level of significance) to detect an association with a hazard ratio of 0.80 in a polymorphism that occurs in 8% of a population, approximately 2200 subjects are required. It is thus no surprise that the results of previous studies had been inconclusive or even contradictory. Time-dependence is also extremely difficult to document in isolated studies. A meta-analysis of individual patients' data [39] offers a useful tool for investigating genetic and other associations in this field.

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Appendix

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